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A PROBLEM OF ACCURACY OF MATHEMATICAL DEAD-TIME ESTIMATION

J. K. HAKEN, M. S. WAINWRIGHT and R. J. SMITH

Departments of Polymer Science and Industrial Chemistry, The University of New South Wales, P.O. Box 1, Kensington 2033 (Australia)

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SUMMARY

The problem of accurate determination of mathematical dead-time is discussed. A comparison of the several methods of calculation is made. The effect of small changes in retention times of *n*-alkanes on the calculated dead-time has been examined and reasons for inaccurate dead-time estimates are suggested.

INTRODUCTION

The presentation of gas chromatographic data in a precise way is important for comparative inter-laboratory studies. Several methods of presentation have been introduced, but the most widely used are the relative retention data method[1] and the retention index scheme of Kováts[2]. Irrespective of the method used, it is necessary to obtain an accurate estimate of the dead-time or column hold-up time. Such estimates may be obtained by direct experimentation or by calculation using retention data of homologs.

Dead-times are determined experimentally by the injection of air, methane or other non-retarded substances[3,4]. Flame ionisation detectors do not normally produce a signal with air. Therefore several methods of calculation of column dead-times have been developed. Of the many classical methods reported, the ones most widely used include: linearisation of the logarithmic plot of homologous *n*-alkane retention times by a graphical trial and error procedure described by Evans and Smith[5], methods using three evenly spaced[6] or consecutive homologs[7], and the method of Gold[8] which permits the use of any three homologs. The last method, which successively approximates the solution of two simultaneous equations, has been adopted for use with Hewlett-Packard HP65 calculator[9].

Several methods of calculation of the dead-time using regression of retention data for homologous alkanes have been described[10,11]. Grobler and Bálizs[10] obtained estimates of the dead-time and the slope and intercept of the regression line of the corrected retention data for *n*-alkanes by the use of two linear regression calculations:

Guardino *et al.*[11] used an iterative technique to find the dead-time and a linear regression to find the slope and intercept in eqn. 1:

$$\ln(t_r - t_m) = bZ + c \tag{1}$$

where t_m is the dead-time, t_r is the uncorrected retention time of the *n*-alkane with carbon number Z, and b and c are the slope and intercept, respectively.

This paper discusses a problem associated with the calculation of mathematical dead-time that became apparent during on-line determination of retention indices.

CALCULATIONS

In order to increase the accuracy and efficiency of determination of retention data, this laboratory has automated a Hewlett-Packard 5750 research chromatograph by interfacing it to a 16K PDP. 11/40 digital computer. Interfacing was achieved by the use of an LPS 11 Laboratory Peripheral System consisting of a 12-bit analog-to-digital convertor, a programmable real-time clock with two Schmitt triggers and a display controller with two 12-bit digital-to-analog convertors. All programming was written in CAPS II Basic with LPS options.

The equipment has been used to measure the Kováts indices of several homologous series of compounds on a number of stationary phases. The retention behaviour of any two different columns was investigated simultaneously by a single injection to a splitter. Frequent determinations of column dead-times were made.

The method of Grobler and Bálizs[10] was adopted for dead-time estimation. Although the authors have claimed this method to be a non-linear regression technique, it in fact involves the calculation of the slopes and intercepts of two linear least squares lines. The values of b, c and t_m in eqn. 1 are calculated by eqns. 2, 3 and 4. These equations are taken from the same paper[10] with the exception that eqn. 2 includes the correction of a mistake in eqn. 1 of the original paper. Grobler has incorrectly used n as the number of t_r values in this equation. There are in fact $(n-1)$ values as given in eqn. 2 below.

$$b = \frac{(n-1)\sum\limits_{i=Z_1}^{Z_{n-1}} Z_i \log(t_{r(i+1)} - t_{r(i)}) - \sum\limits_{i=Z_1}^{Z_{n-1}} Z_i \sum\limits_{i=Z_1}^{Z_{n-1}} \log(t_{r(i+1)} - t_{r(i)})}{(n-1)\sum\limits_{i=Z_1}^{Z_{n-1}} Z_i^2 - \left(\sum\limits_{i=Z_1}^{Z_{n-1}} Z_i\right)^2} \tag{2}$$

$$t_m = \frac{\sum\limits_{i=Z_1}^{Z_n} q^{Z_i} \sum\limits_{i=Z_1}^{Z_n} t_{r(i)} \cdot q^{Z_i} - \sum\limits_{i=Z_1}^{Z_n} q^{2Z_i} \sum\limits_{i=Z_1}^{Z_n} t_{r(i)}}{\left(\sum\limits_{i=Z_1}^{Z_n} q^{Z_i}\right)^2 - n \sum\limits_{i=Z_1}^{Z_n} q^{2Z_i}} \tag{3}$$

$$c = \frac{\sum\limits_{i=Z_1}^{Z_n} \log t'_{r(i)} - b \sum\limits_{i=Z_1}^{Z_n} Z_i}{n} \tag{4}$$

where $t_{r(i)}$ is the uncorrected retention time of the i-th n-alkane, $t'_{r(i)}$ is the corrected retention time of the i-th n-alkane, Z_i is the carbon number of the i-th n-alkane, q is antilog b, and $n =$ number of n-alkanes used. Thus the retention index for any compound can be calculated by

$$I = 100 \, (\log t'_r - c)/b \tag{5}$$

When determining I values on a 12 ft. $\times$ $^{1}/_{4}$ in. column packed with 10% squalane and operating at 120° it became apparent that small differences (of the order of 2 sec) in uncorrected retention times of C_7–C_{10} alkanes led to large differences (up to 10 sec) in t_m values. Typical results are given in Table I.

TABLE I

VARIATION OF DEAD-TIMES FOR RUNS USING ALKANES C_7–C_{10}

Run	Uncorrected retention time				Calculated dead-time
	C_7	C_8	C_9	C_{10}	
1	182	291	497	887	59.77
2	182	291	497	889	60.50
3	182.5	290	495.5	884	63.04
4	184	290	494.5	888.5	69.75

RESULTS AND DISCUSSION

Table I shows the effect of small changes in uncorrected retention times of n-alkanes on the value of t_m determined by the method of Grobler and Bálizs[10]. Table II compares the values obtained by their methods with the four other methods.

Eqn. 1 is non-linear in the parameters t_m, b and c. The best estimates of these parameters would normally be obtained by non-linear least squares regression. However, by using the differences in retention times between adjacent n-alkanes Grobler and Bálizs have simplified the problem to two linear regression calculations. The slope of the corrected retention time line is obtained first by eqn. 2 and the dead-time is calculated independently using eqn. 3. In this study we have compared the t_m and b values calculated by Grobler and Bálizs' method, by two iterative methods and by two direct calculation methods[6,8].

The method of Guardino et al.[11] uses an iterative technique to calculate t_m and a linear least squares method to evaluate b and c. The best estimates of t_m and b and c are obtained by minimising the sum of squares of the differences between the calculated and actual I values for the alkanes.

The second iterative method used was a non-linear regression to calculate t_m, b and c simultaneously by minimising the sum of squares of the predicted and actual I values. The minimisation was effected by the use of the Flexible Simplex method of optimisation described by Spendley et al.[12] and modified by Nelder and Mead[13].

It is obvious from the closeness of the sum of squares and t_m values that the simpler method of Grobler and Bálizs is almost as accurate as the two iterative procedures. The values of t_m calculated by direct methods are only slightly different from

TABLE II

COMPARISON OF DIFFERENT METHODS FOR CALCULATING DEAD-TIMES

Methods: A = Non-linear regression using simplex; B = method of Grobler and Bálizs[10]; C = method of Guardino et al.[11]; D = method of Peterson and Hirsch[6]; E = method of Gold[8].

Run	Method				
	A	B	C	D	E
Dead-times					
1	59.79	59.77	59.74	59.95	59.95
2	60.58	60.50	60.53		
3	62.91	63.04	62.86		
4	69.74	69.75	69.73	69.62	69.62
Slopes					
1	0.6374	0.6374	0.6373		
2	0.6400	0.6400	0.6398		
3	0.6424	0.6424	0.6423		
4	0.6565	0.6565	0.6564		
Sum of squares					
1	1.394×10^{-3}	1.728×10^{-3}	1.500×10^{-3}		
2	2.157×10^{-2}	2.665×10^{-2}	2.171×10^{-2}		
3	5.665×10^{-2}	7.059×10^{-2}	5.675×10^{-2}		
4	8.280×10^{-4}	1.041×10^{-3}	8.348×10^{-4}		

those obtained using the regression methods. Furthermore, it is apparent that the method of calculation does not account for the large differences in t_m values obtained when there are small changes in uncorrected retention times of the alkanes.

The principle of the calculation of mathematical dead-time involves the extrapolation of the least squares line through the logarithms of the corrected retention times to zero carbon number. Therefore small changes in the slope of this line will lead to large variations in calculated values of t_m and hence to large variations in I values[11]. In order to quantify the effect of small systematic changes in retention times of the *n*-alkanes, small perturbations (of the order of 1 or 2 sec) were made to

TABLE III

EFFECT OF SMALL PERTURBATIONS IN RETENTION TIMES

Alkane	Experimental data	Perturbed Data			
	Retention time	Retention time	Deviation	Retention time	Deviation
C_5	86	84	−2	88	+2
C_6	110	109	−1	111	+1
C_7	156	157	+1	155	−1
C_8	245	247	+2	243	−2
Dead-time	60.13	55.60	−4.53	64.18	+4.05
C_7	182	180	−2	184	+2
C_8	291	290	−1	292	+1
C_9	497	498	+1	496	−1
C_{10}	887	889	+2	885	−2
Dead-time	59.79	55.60	−4.19	63.98	+4.17

TABLE IV

RETENTION AND DEAD-TIME MEASUREMENTS MADE ON SQUALANE AND OV-7 COLUMNS

Uncorrected retention time				Dead-time
C_7	C_8	C_9	C_{10}	
Squalane				
182	291	497	887	59.77
184	290	494.5	888.5	69.75
OV-7				
113.5	150.5	216.5	335.5	66.93
113	150	215	332.5	65.52

uncorrected retention data for two sets of alkanes, C_5–C_8 and C_7–C_{10}. These results are presented in Table III. It is obvious from these results that small changes in the alkane retention times lead to large changes in the dead-time value.

Of the several stationary phases investigated squalane was the only one to give significant differences in dead-time values. Table IV presents typical values of dead-time for an OV-7 column that was coupled to the squalane column via a splitter. The results show that although the retention times of the alkanes do show variations of similar magnitude on both columns, the differences in dead-time for OV-7 are significantly less. It should be noted that the mean dead-time for squalane over a large number of determinations was approximately 60 sec whilst the value for OV-7 was approximately 67 sec. The greater inaccuracy in the dead-time determination for squalane appeared to be associated with the relatively long retention times of the C_7–C_{10} alkanes. Therefore C_5–C_8 alkanes were used and Table V lists the dead-time values calculated over a 6-h period of Kováts' index calculations. It can be seen that the variation in dead-time is not significant.

It is apparent from the results of Tables IV and V that significant differences in dead-time result from using alkanes having long retention times. Longer retention times obviously mean a further extrapolation and a larger error. Therefore it is desirable to use low-molecular-weight alkanes provided retention times can be measured with sufficient accuracy. However, it should be noted that low homologues generally give non-linear plots of log t'_r vs. carbon number. In this study the retention times were measured to 0.5-sec accuracy using the real-time clock in the computer.

TABLE V

EFFECT ON MATHEMATICAL DEAD-TIME WHEN C_5–C_8 ARE USED

Uncorrected retention time				Dead time
C_5	C_6	C_7	C_8	
85	108	152	236.5	59.92
85.5	109	153	239	59.88
85.5	108.5	153	238.5	60.70
86.0	110	156.5	246	60.21
86	110	156	244.5	59.93
86	110	156	245	60.10

The significant differences in dead-times computed from retention times of higher alkanes may be attributed to the fact that fluctuations in column operating conditions will have a greater effect on substances that are retained for longer periods. Changes in retention time of the order of ± 1 sec in 400 sec may be attributed to extremely small fluctuations in column temperature. Mikkelsen[14] reports that a change of 1° in column temperature can cause a variation of 2.5 % in retention time. Therefore changes in temperature of $\pm 0.1°$ can produce variations in retention time of ± 1 sec at retention times of the order of 400 sec. Any determination of mathematical dead-time must be treated with caution since incorrect dead-times have a marked influence on the Kováts I value.

CONCLUSIONS

It has been observed that mathematical dead-time calculations are markedly influenced by small changes in retention times of *n*-alkanes. A judicious selection of the alkane mixture to be used as a standard is required to obtain accurate dead-time values. Several methods of calculating dead-time have been investigated and a simple method using linear least squares analysis has been shown to be as accurate as iterative techniques.

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